REMARKS

This submission is in response to the Official Action dated November 5, 2002. Claims 156-159, 164, 167, 175, 179, 183, and 187 have been amended. Claims 174, 178, 182, and 186 have been cancelled, without prejudice or disclaimer. Accordingly, claims 146-173, 175-177, 179-181, 183-185, and 187-189 are pending and at issue. For the Examiner's convenience, a document showing all pending and allowed claims, as amended herewith, is enclosed ("Examiner's Courtesy Copy"). Reconsideration of the above identified application, in view of the above amendments and the following remarks, is respectfully requested.

Claim 161, 164 and 167 have been amended to remove repetitive subject matter from the preamble of each respective claim. Specifically, the phrase "having at least 90% sequence identity to SEQ ID NO:2" has been removed from claims 161 and 164, and the phrase "comprising a mutation at a position corresponding to at least one of amino acid 331, 280, and 242 of cytochrome $P450_{cam}$ from *P. putida* (SEQ ID NO:2) and having at least 90% sequence identity to SEQ ID NO:2" has been removed from claim 167, since the same test enzyme features are recited in step (d) of the claims.

Claims 156-159 have been amended to incorporate the subject matter of claims 174, 178, 182, and 186, respectively, into the claims. Specifically, as amended, claims 156-159 recite that the cytochrome P450 oxygenase variant has

a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2. The same limitation has also been added to claim 161.

Claims 175, 179, 183, and 187 have been amended to depend from claims 156-159 instead of from canceled claims.

Rejection Under 35 U.S.C. §112, 1st Paragraph

The Examiner has rejected claims 156-159, 176, 177, 180, 181, 184, 185, 188, and 189 for alleged lack of written description. Specifically, the Examiner contends that because the claims are directed to a genus of polypeptides, no description has been provided of all the modified polypeptides encompassed by the claims (Office Action, page 3).

Applicants respectfully disagree. With this response, claims 156-159 have been amended to recite that the cytochrome P450 oxygenase variant has a mutation in at least one position corresponding to at least one of amino acids 242, 280, and 331 of SEQ ID NO:2, thus providing a clear defining structural feature for all of the polypeptides of claims 156-159. Claims 176, 177, 180, 181, 184, 185, 188, and 189 depend from claims 156-159, thereby incorporating all limitations of these claims. On page 55 of the specification, three exemplary mutants having various combinations of the claimed mutations in SEQ ID NO:2 are shown. These mutants display increased oxidation activity towards exemplary substrates such as 3-phenylpropionate, coumarin, and naphthalene, (see also p. 54, Table 2). For

example, one particular mutant (M7-6H), having mutations in positions 280 and 331, had about 11 times higher oxidation activity of naphthalene than the wild-type enzyme (p. 55, lines 4-6).

Accordingly, applicants were in possession of the subject matter of claims 156-159, 176, 177, 180, 181, 184, 185, 188, and 189 at the time of filing of the application. Reconsideration and withdrawal of this rejection, in view of the above arguments and amendments, is respectfully requested.

Rejection Under 35 U.S.C. §102

Claims 156, 176, and 177 have been rejected as allegedly anticipated by England et al. (FEBS Letts 1998;424:271-274). The Examiner contends that the England et al. reference reports a $P450_{cam}$ variant with a oxidation rate about 1 to 2 orders higher than the wild-type enzyme.

Applicants respectfully disagree. As amended, claims 156, 176, and 177 all recite $P450_{cam}$ mutants having at least one mutation at a position corresponding to one of positions 242, 280, and 331 in SEQ ID NO:2. England et al. does not disclose a mutation in any of these residues. In fact, if the amino acid sequence of England et al.'s Y96 mutant was to be aligned with the amino acid sequence of the variant enzyme of the invention, the Y96 residue would be nowhere near the mutation sites of the presently claimed invention, those corresponding to positions 242, 280, and 331 in SEQ ID NO:2.

Accordingly, reconsideration and withdrawal of this rejection, in view of the above arguments and amendments, is respectfully requested.

Rejection Under 35 U.S.C. 103

Claims 156-159, 161-166, 176-177, 180-181, 184-185, 188-189 have been rejected as allegedly obvious over Kuchner et al. (TIB Tech 1997;15:523-30), England et al. (FEBS letters 1998;424:271-274), and Wong et al. (WO 97/16553). The Examiner contends that the combination of these three references would have made it obvious to plan and evolve $P450_{cam}$ such that the mutant is at least two or ten times more active and stable than the wild-type. The Examiner also contends that England and Wong provides a reasonable expectation of success in developing such mutants.

Applicant's respectfully disagree. The Kuchner et al. reference describes directed evolution techniques. Wong et al. describes $P450_{cam}$ mutants having point mutations/deletions in the active site, some of which have an improved turnover rate (measured as NADPH consumption; see Table 3). The England et al. reference is discussed above. There are several reasons why these references, alone or in combination, fail to render obvious any one of claims 156-159 and 161, or any claims dependent thereon.

First, there is no motivation to combine the directed evolution methods of Kuchner et al. with the modeling methods of England or Wong to arrive

at the presently claimed invention. England and Wong both select particular residues believed to be important for the oxidation activity of the wild-type enzyme and replace the amino acid residue in question with another amino acid, while Kuchner et al. produces a library of enzyme variants by inducing random mutations.

Second, none of these references describe cytochrome P450 variants having a mutation in a position corresponding to amino acids 242, 280, or 331 of SEQ ID NO:2, a feature recited in all of claims 156-159 and 161. Kuchner et al. does not describe any specific mutations in any enzyme, the mutation described in the England et al. reference is nowhere near the mutations of the present invention (see above), and the Wong et al. PCT publication does not consider mutations at residues 242, 280 or 331 of $P450_{cam}$. According to the MPEP, section 2143.03, "[t]o establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

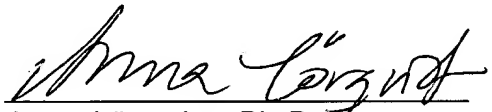
Claims 162-166, 176-177, 180-181, 184-185, 188-189 depend directly or indirectly from claims 156-159 or 161. If an independent claim is nonobvious under 35 U.S.C. §103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071,5 USPQ2d 1596 (Fed. Cir. 1988). Accordingly, all of claims 156-159, 161-166, 176-177, 180-181, 184-185, 188-189 are non-obvious over the cited combination of prior art references. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

* * *

Accordingly, in view of the above amendments and remarks, it is earnestly believed that all of the pending claims are in condition for allowance, and that the case can be passed to issue.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



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Limited Recognition Under 37 C.F.R. §10.9(b) (see attached)
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Frances H. ARNOLD, et al.

Serial No.: 09/246,451 Art Unit: 1652

Confirmation No.: 6181

Filed: February 9, 1999 Examiner: Manjunath N. RAO

For: OXYGENASE ENZYMES AND SCREENING METHOD

MARK -UP FOR RESPONSE TO OFFICIAL ACTION

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

April 7, 2003

Sir:

156. (Twice amended) A cytochrome P450 oxygenase variant having a catalytic activity at least two times the catalytic activity of wild-type cytochrome $P450_{cam}$ oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, [and] at least 90%

sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

157. (Twice amended) A cytochrome P450 oxygenase variant having a catalytic activity at least about ten times the catalytic activity of wild-type cytochrome $P450_{cam}$ oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, [and] at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

158. (Twice amended) A cytochrome P450 oxygenase variant having a stability at least two times the stability of wild-type cytochrome $P450_{cam}$ oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, [and] at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

159. (Twice amended) A cytochrome P450 oxygenase variant having a stability at least about ten times the stability of wild-type cytochrome $P450_{cam}$ oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, [and] at least 90%

sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

161. (Twice amended) An oxygenase variant evolved from a wild-type oxygenase enzyme, and having a catalytic activity at least ten times the catalytic activity of the wild-type oxygenase enzyme in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor [and at least 90% sequence identity to SEQ ID NO:2], which oxygenase variant was identified by a method comprising the steps of:

(a) contacting a test enzyme variant with an oxygenase substrate and the oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme;

(b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product;

(c) detecting the detectable composition; and

(d) selecting any test enzyme having at least 10 times the catalytic activity of the wild-type oxygenase enzyme in the presence of the oxygen donor , [and] at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

164. (Amended) An oxygenase variant evolved from a wild-type oxygenase enzyme, and having a stability at least ten times the stability of the wild-type oxygenase enzyme in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor [and at least 90% sequence identity to SEQ ID NO:2], which oxygenase variant was identified by a method comprising the steps of:

(a) contacting a test enzyme variant with an oxygenase substrate and the oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme;

(b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product;

(c) detecting the detectable composition; and

(d) selecting any test enzyme having at least 10 times the stability of the wild-type oxygenase enzyme, [and] at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to at least one of amino acids 242, 280, and 331 of SEQ ID NO:2.

167. (Amended) A functional cytochrome P450 oxygenase variant [comprising a mutation at a position corresponding to at least one of amino acid 331, 280, and 242 of cytochrome $P450_{cam}$ from *P. putida* (SEQ ID NO:2) and having at least 90% sequence identity to SEQ ID NO:2, which cytochrome P450 oxygenase variant was] identified by a method comprising the steps of:

(a) contacting a test cytochrome P450 oxygenase variant with an oxygenase substrate and an oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme;

(b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product;

(c) detecting the detectable composition; and

(d) selecting any test enzyme having a mutation at a position corresponding to at least one of amino acid 331, 280, and 242 of cytochrome $P450_{cam}$ from *P. putida* (SEQ ID NO:2) and at least 90% sequence identity to SEQ ID NO:2.

175. (Amended) The cytochrome P450 variant of claim [174] 156, comprising at least one mutation selected from lysine at amino acid 331, leucine at amino acid 280, and phenylalanine at amino acid 242.

179. (Amended) The cytochrome P450 variant of claim [178] 157, comprising at least one mutation selected from lysine at amino acid 331, leucine at amino acid 280, and phenylalanine at amino acid 242.

183. (Amended) The cytochrome P450 variant of claim [182] 158, comprising at least one mutation selected from lysine at amino acid 331, leucine at amino acid 280, and phenylalanine at amino acid 242.

187. (Amended) The cytochrome P450 variant of claim [186] 159, comprising at least one mutation selected from lysine at amino acid 331, leucine at amino acid 280, and phenylalanine at amino acid 242.